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Form PTO-1390		U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER TUR-125
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U S C 371		U S APPLICATION NO 10/069145	
INTERNATIONAL APPLICATION NO PCT/FI00/00710	INTERNATIONAL FILING DATE August 22, 2000	PRIORITY DATE CLAIMED August 25, 1999	
TITLE OF INVENTION NOVEL COMPOSITIONS FOR CONTROLLED RELEASE OF A BIOLOGICALLY ACTIVE AGENT, AND THE PREPARATION THEREOF		DATE February 22, 2002	
APPLICANT(S) FOR DO/EO/US Manja AHOLA, Eija SAILYNOJA, Jukka SALONEN, Risto PENTTINEN and Antti YLI-URPO			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. <input type="checkbox"/> This a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1) <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau) <input checked="" type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau) <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired <input checked="" type="checkbox"/> have not been made and will not be made. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)) <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 			
Items 11. to 16. below concern other document(s) or information included:			
<ol style="list-style-type: none"> <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. <input type="checkbox"/> A substitute specification <input type="checkbox"/> A change of power of attorney and/or address letter. <input checked="" type="checkbox"/> Other items or information: <ol style="list-style-type: none"> WO 01/13924 International Search Report (PCT/ISA/210) International Search Report (Revised) International Preliminary Examination Report (PCT/IPEA/409) 			

U.S. Application No 10/069145		International Application No PCT/FI00/00710		Attorney's Docket No TUR-125	
17. [XX] The following fees are submitted				CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO. \$890.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) \$710.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$740.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$ 1040.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$ 100.00				\$ 1040.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$ 1,040.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	9 - 20	0	x \$ 18.00	\$	
Indep. claims	1 - 3	0	x \$ 84.00	\$	
Multiple dependent claim(s) (if applicable)			+ \$280.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$ 1,040.00	
Reduction by 1/2 for filing by small entity, if applicable (Note 37 CFR 1.9, 1.27, 1.28)				\$ 520.00	
SUB TOTAL =				\$ 520.00	
Processing fee \$130.00 for furnishing the English translation later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$ 520.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) \$40.00 per property +				\$ 40.00	
TOTAL FEES ENCLOSED =				\$ 560.00	
				Amount to be: refunded	\$
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<p>a. [XX] A Credit Card Payment Form or check in the amount of \$ <u>560.00</u> to cover the above fee is enclosed</p> <p>b. [] Please charge my Deposit Account No. <u>50-1258</u> in the amount of \$ _____ to cover the above fees Two copies of this sheet are enclosed</p> <p>c. [XX] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>50-1258</u> Two copies of this sheet are enclosed.</p> <p>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to review (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</p>					
SEND ALL CORRESPONDENCE TO James C. Lydon 100 Daingerfield Road Suite 100 Alexandria, Virginia 22314				Signature _____ James C. Lydon Name _____ 30,082 Registration Number _____ 2/22/02 Date _____	

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Manja AHOLA et al.

Serial Number: New Application

Filed: February 22, 2002

For: NOVEL COMPOSITIONS FOR CONTROLLED RELEASE OF A BIOLOGICALLY
ACTIVE AGENT, AND THE PREPARATION THEREOF

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

February 22, 2002

Sir:

Prior to calculation of the filing fee, please amend this
application as follows:

IN THE SPECIFICATION:

Page 1, between the title and line 4, please insert the
following:

This application is a U.S. national stage of International
Application PCT/FI00/00710, filed August 22, 2000 and published on
March 1, 2001 in the English language.

IN THE CLAIMS:

Please cancel claims 1-7 without prejudice or disclaimer.

Please add new claims 8-16 as follows:

8. (New) A composition for controlled release of a biologically
active agent from a carrier, wherein the biologically active agent
is heparin or a related biologically active acidic polysaccharide,

New U.S. Nat'l Stage Application
PRELIMINARY AMENDMENT

PATENT

and wherein the carrier is a sol-gel derived silica xerogel, the xerogel is derived from a tetraalkoxysilane and that part of the tetraalkoxysilane is replaced by an organomodified alkoxysilane.

9. (New) The composition of claim 8, wherein said tetraalkoxysilane is tetraethoxysilane (TEOS), and said organomodified alkoxysilane is an alkylsubstituted alkoxysilane.

10. (New) The composition of claim 9, wherein said alkylsubstituted alkoxysilane is a member selected from the group consisting of methyltriethoxysilane (METES), dimethyldiethoxysilane (DMDES) and ethyltriethoxysilane (ETES).

11. (New) The composition of claim 8, wherein said biologically active agent is heparin and which is present in an amount of 5 to 30 weight percent, calculated on the air dried xerogel.

12. (New) A method for the preparation of a composition of claim 8, comprising

- a) hydrolysing an alkoxysilane and an organomodified alkoxysilane in the presence of a catalyst,
- b) optionally adjusting the pH to a value suitable for the biologically active agent,

New U.S. Nat'l Stage Application
PRELIMINARY AMENDMENT

PATENT

c) adding the biologically active agent,
d) allowing the hydroxysilane to polymerize, and optionally
e) removing water and alcohol formed in the hydrolyzation from the mixture.

13. (New) The method of claim 12, wherein the alkoxysilane is a tetraalkoxysilane.

14. (New) The method of claim 12, wherein the organomodified alkoxysilane is an alkylsubstituted alkoxysilane.

15. (New) The method of claim 14, wherein said alkylsubstituted alkoxysilane is at least one member of the group consisting of methyltriethoxysilane (METES), dimethyldiethoxysilane (DMDES) and ethyltriethoxysilane (ETES).

16. (New) The method of claim 12, wherein nitric acid or acetic acid is used as a catalyst.

IN THE ABSTRACT:

Please insert the attached Abstract into the application after the claims.

New U.S. Nat'l Stage Application
PRELIMINARY AMENDMENT

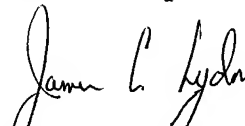
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REMARKS

This Preliminary Amendment cancels claims 1-7, adds new claims 8-16, inserts a reference to International Application PCT/FI00/00710 into the specification, and presents a new Abstract based on the PCT Abstract. The new claims correspond to the original claims, but do not contain multiple dependencies. A version showing the changes made is attached as an Appendix. Claims 8-16 are pending.

It is not believed that any fee is required for entry and consideration of this Preliminary Amendment. Nevertheless, the Commissioner is authorized to charge our Deposit Account No. 50-1258 in the amount of any such fee deemed necessary for such entry and consideration.

Respectfully submitted,


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Enclosures:
Appendix
Abstract

New U.S. Nat'l Stage Application
PRELIMINARY AMENDMENT

PATENT

Appendix
Version With Markings Showing Changes

IN THE SPECIFICATION:

Page 1, the paragraph between the title and line 4 is new.

IN THE CLAIMS:

Claims 1-7 have been canceled.

Claims 8-16 are new.

IN THE ABSTRACT:

The attached Abstract is new.

ABSTRACT OF THE DISCLOSURE:

A composition for controlled release of a biologically active agent from a carrier. The biologically active agent is heparin or a related biologically active acidic polysaccharide and the carrier is a sol-gel derived silica xerogel. The xerogel is derived from a tetraalkoxysilane such as tetrethoxysilane (TEOS) and part of the tetraalkoxysilane is replaced by an organomodified alkoxysilane, preferably an akylsubstituted alkoxysilane. The invention also includes a method for the preparation of this composition.

NOVEL COMPOSITIONS FOR CONTROLLED RELEASE OF A
BIOLOGICALLY ACTIVE AGENT, AND THE PREPARATION THEREOF

- 5 This invention concerns a composition for the controlled release of a biologically active agent from a carrier, and the preparation of said composition.

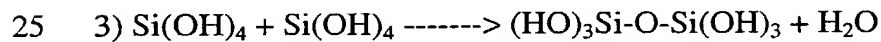
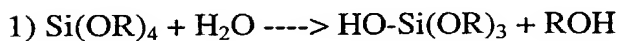
BACKGROUND OF THE INVENTION

- 10 The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference.

- By xerogel is meant a dried gel. Silica xerogels are partly hydrolyzed oxides of
15 silicium. Hydrolyzed oxide gels can be produced by a sol-gel process, which has been used for producing ceramic and glass materials for several years.

The sol-gel process is based on hydrolyzation of a metal-alkoxide and subsequent polymerization of the metal hydroxides as follows:

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- As the polymerization reaction progresses, additional chains, rings and three-dimensional networks are formed, and a gel, comprising water, the alcohol of the alkoxy group and the gel itself, is formed. The sol may also contain other additives,
30 such as acids or bases, which are used as catalysts for the reaction. Further additives

such as polyethylene glycol (PEG) can also be added to influence on the porosity of the gel. If alcohol and water are now extracted from the gel by washing and evaporation, a xerogel is obtained.

- 5 The polymerization of the remaining OH groups continues during the drying. The polymerization continues for a long time even after the gelation. This is called ageing. The further the polymerization proceeds, the more stable the gel or xerogel becomes. At room temperature, however, the polymerization will in fact stop after an ageing of a few weeks, and the xerogel will not become completely inert. If the
10 temperature is raised, the polymerization reaction can be accelerated, the gel becomes more stable and shrinkage occurs, and internal stresses appear in the xerogel to an increasing degree.

The controlled release of therapeutic agents from biodegradable matrix has become
15 increasingly important for implantable delivery systems, due to its advantages of safety, efficacy and patient convenience. The sol-gel technique offers new possibilities for incorporating biologically active agents within silica xerogels at room temperature process, and for controlling their release rates from silica xerogel matrix in time dependent manner (Nicoll et al. 1997; Ahola et al. 1998; Böttcher et
20 al. 1998; Kortesus et al., 1998; Sieminska et al., 1996). This sol-gel technology is cheap, versatile and simple, and silica xerogels produced by this technique are biocompatible and non-toxic materials (Kortesus et al. 1998; Radin et al. 1998; Kortesus et al. 1999). Earlier studies have shown that chemical and physical
25 changes into the silica xerogel matrix have effect on the releasing behavior of biologically active agents because of the drug release from silica xerogel is the combined process of diffusion and matrix erosion.

A major concern with the use of artificial organs and biomedical devices is the
untoward interactions of blood upon contacting a foreign surface. The most obvious
30 complications are those related to the haemostatic mechanism, which can lead to

thrombus formation and impaired function or occlusion of medical devices.

Intravascular stenting is often used after angioplasty to prevent a reocclusion of the damaged vessel following dilatation. One problem inherent to stent implantation is a possible restenosis. The process of restenosis is attributed to myointimal

5 hyperplasia as well as to thrombus formation (Palmaz, 1993, Van Beusekom et al., 1993). The interaction of platelets with the stent surface may have significance not only due to their involvement in thrombus formation, but also by the release of platelet derived growth factor that may be included in the stimulation of smooth muscle cell growth (Palmaz, 1993, Ross, 1986). Heparin is routinely used for the
10 prophylaxis of both surgical and medical thrombosis.

However, there is no disclosure or suggestion in prior art indicating that compositions for the controlled release of heparin could be achieved by incorporating heparin in a sol-gel derived silica xerogel, and that such a

15 composition would be useful for treating and/or preventing thrombosis. Known heparin preparations are administered as injections. Thus, there is a great need for more convenient administration routes of heparin, especially for long acting, controlled release dosage forms of heparin.

20 OBJECTS AND SUMMARY OF THE INVENTION

The aim of this invention is to provide a composition for the controlled release of heparin or a related biologically active acidic polysaccharide , wherein said composition can be used for systemic or local prophylaxis and/or treatment of
25 medical or surgical thrombosis.

Another object is to provide a method for the preparation of a composition for the controlled release of heparin or a related biologically active acidic polysaccharide .

Thus, according to one aspect, this invention concerns a composition for controlled release of a biologically active agent from a carrier, wherein the biologically active agent is heparin or a related biologically active acidic polysaccharide and the carrier is a sol-gel derived silica xerogel. The xerogel is derived from a tetraalkoxysilane such as tetrethoxysilane (TEOS) and part of the tetraalkoxysilane is replaced by an organomodified alkoxysilane, preferably an alkylsubstituted alkoxysilane.

According to another aspect, this invention concerns a method for the preparation of a composition according to this invention. The method is characterized by the steps of

- a) hydrolyzing an alkoxysilane and an organomodified alkoxysilane in the presence of a catalyst,
- b) optionally adjusting the pH to a value suitable for the biologically active agent,
- c) adding the biologically active agent,
- d) allowing the hydroxysilane to polymerize, and optionally
- e) removing water and alcohol formed in the hydrolyzation from the mixture.

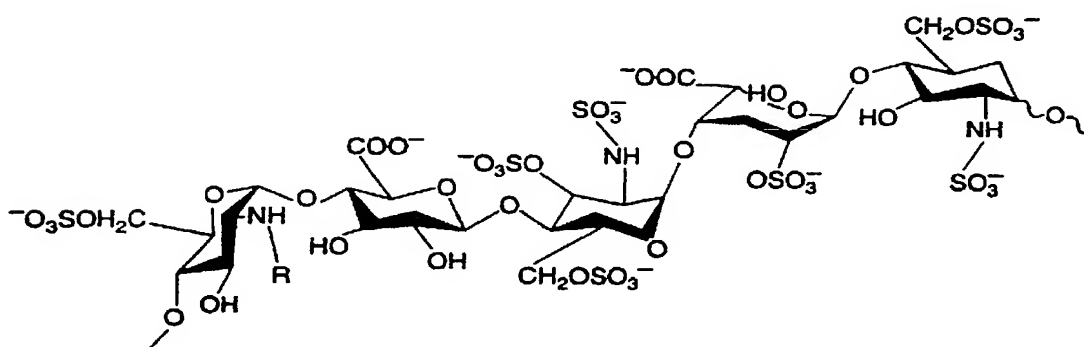
BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the cumulative release of heparin versus time for formulations containing 1 weight-% of heparin, calculated on the sol, for xerogels made using nitric acid (open squares) or acetic acid (filled squares) as catalyst.

Figure 2 shows the cumulative release of heparin in percent versus time for formulations containing 1, 1.5, 2, 3 and 4 weight-% of heparin, calculated on the sol, for xerogels made using acetic acid as catalyst.

DETAILED DESCRIPTION OF THE INVENTION

Heparin is a linear polysaccharide containing repeated units of six sugar residues, each consisting of an alternating sequence of sulfate derivatives on N-acetyl-D-glucosamine and D-iduronate (formula I). Heparin is a powerful anticoagulant and it is also a component of the extracellular matrix of blood vessels and promotes endothelial cell growth *in vitro*.



(I)

According to this invention, heparin can alternatively be replaced by a related biologically active acidic polysaccharide. As examples of such acidic polysaccharides having antithrombotic effects can be mentioned heparan sulfate proteoglycan, sulfonated hyaluronic acid and the like.

The heparin or the related biologically active acidic polysaccharide can either be of natural origin or biotechnically manufactured.

The purpose of the present study was to evaluate the suitability of sol-gel produced silica xerogel as the carrier matrix for controlled release of heparin or a related acidic polysaccharide. The influence of sol-gel parameters, such as catalysts or various alkoxysiloxanes, and the effect of heparin concentration were studied. Also the maintenance of biological activity of the drug after sol-gel process was tested. The release of heparin was linear according to zero order kinetics, and the release

rates of different matrixes were found to be directly proportional to the drug load of the matrix. The release rate can be controlled by choosing the used catalyst in the sol-gel process. Other parameters affecting the structure and properties of the silica xerogels, such as the release rate of the drug, are the temperature, pH, drying and heating conditions of the silica sol. Also by chemical modification of silica xerogel network the release rate of heparin can be controlled.

The xerogel is derived from a tetraalkoxysilane such as tetraethoxysilane (TEOS). In case more brittle xerogels are desired, part of the tetraalkoxysilane (e.g. TEOS) is replaced by an organomodified alkoxysilane, preferably an alkylsubstituted alkoxysilane. As particularly preferred alkylsubstituted alkoxysilanes can be mentioned methyltriethoxysilane $\text{MeSi}(\text{OEt})_3$ (METES), dimethyldiethoxysilane $\text{Me}_2\text{Si}(\text{OEt})_2$ (DMDES) or ethyltriethoxysilane $\text{EtSi}(\text{OEt})_3$ (ETES). In case about 25 % of the amount of TEOS is repalced by one of the aforementioned organomodified alkoxysilanes, an increased release rate of the drug can be foreseen.

The amount of heparin is preferably about 5 to 15 weight-% calculated on the air dried xerogel.

Nitric acid or acetic acid is preferably used as catalyst.

The composition can be used for the treatment and/or prevention of surgical or medical thrombosis, for local or systemic use. Among the preferable administration routes can be mentioned subcutaneous or intramuscular dosage forms. Also long-acting injection forms could be prepared because the heparin loaded xerogel can be finished into small, injectable particles. According to a preferable embodiment, the formulation is an implantate to be placed in the close vicinity of the object undergone surgical operation. The formulation can also be used during the operation.

The invention will be described more in detail in the Experimental section in the following non-limiting examples.

Experimental

5

Preparation of silica sol

The silica sol loaded with heparin was prepared by a two step sol-gel process using acid as a catalyst (Ellerby *et al.* 1992). The following reagents were used,

- 10 tetraethoxysilane (TEOS) (Aldrich), deionized water, nitric acid (HNO₃) (Merck), acetic acid (CH₃COOH) (Merck), ammonium hydroxide (NH₄OH) (Merck) and heparin sodium salt (Orion Corporation, Finland). The biological activity of the used heparin was 84 IU/mg measured by Factor Xa assay (HEPRN). The first step of the reaction series was a hydrolysis reaction between water and alkoxide. The
- 15 mol ratio of the silica sol was TEOS:H₂O:HNO₃ = 1 : 15 : 0.0015 and TEOS:H₂O:CH₃COOH = 1 : 15 : 0.026, respectively. Modification of the nitric acid catalyzed sol was carried out by co-hydrolysis of TEOS with the following organomodified (i.e. alkylsubstituted) alkoxysilanes: dimethyldiethoxysilane Me₂Si(OEt)₂ (DMDES) (Lancaster), methyltriethoxysilane MeSi(OEt)₃ (METES)
- 20 (Aldrich) or ethyltriethoxysilane EtSi(OEt)₃ (ETES) (Lancaster). For the partial substitution of TEOS, 10 or 25 mol-% organomodified alkoxysilane was used. After the first step, i.e. hydrolysis reaction, pH was raised to 4.5-4.8 with base (0.1 or 1 M NH₄OH) before heparin addition. The heparin sodium salt was first dissolved in the deionized water and then added to the hydrolysis solution. The
- 25 concentration of heparin in silica sol ranged from 1 wt % to 4 wt % calculated on the sol, corresponding to 6.8 - 29.2 wt % in the air dried silica xerogel. The silica sol was cast into Blister plate wells, kept at 40°C and 40% relative humidity for polycondensation and ageing. The aged silica gels were dried at 40°C and 40% relative humidity to constant weight to obtain silica xerogels containing
- 30 incorporated heparin. The formulations prepared are disclosed in Table 1.

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Table I

Formulation parameters used in the study.

Formulation no.	Silane (mol-%)				Catalyst		Heparin conc. in the sol (wt %)
	TEOS	METES	ETES	DEDMS	acetic acid	nitric acid	
1	100				x		1
2	100				x		1.5
3	100				x		2
4	100				x		3*
5	100				x		4*
6	100					x	1
7	90	10				x	2
8	75	25				x	2
9	90		10			x	2
10	75		25			x	2
11	90			10		x	2
12	75			25		x	2

* water/TEOS ratio = 24

In vitro* release experiments*Dissolution test**

- 5 The dissolution profiles of heparin and silica from the silica xerogel matrixes were studied in a shaking water bath at 37 °C. Simulated body fluid (SBF) was used as a dissolution medium. SBF was prepared by dissolving reagent grade NaCl, NaHCO₃, KCl, K₂HPO₄ x 3H₂O, MgCl₂ x 6H₂O, CaCl₂ x 2H₂O, Na₂SO₄ in deionized water (Table 2). The solution was buffered with
- 10 tris(hydroxymethyl)aminomethane (TRIZMA) and hydrochloride acid (HCl) at physiological pH 7.40. The composition of inorganic ions emulated that of human blood plasma.

Table 2

Reagent	concentration (mM)
NaCl	136.8
NaHCO ₃	4.2
KCl	3.0
K ₂ HPO ₄ x 3H ₂ O	1.0
MgCl ₂ x 6H ₂ O	1.5
CaCl ₂ x 2 H ₂ O	2.5
Na ₂ SO ₄	0.5
TRIZMA	50

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The silica xerogel sample was immersed in 50 ml SBF in a polyethylene bottle covered with a tight lid. Alternately, 5 ml sample or the whole medium was withdrawn from each flask and replaced immediately with fresh medium. Three parallel samples were used.

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Toluidine blue test

The total amount of heparin dissolved was measured by a colorimetric toluidine blue method (Smith et al., 1980) modified to our purposes. 0.005 % toluidine blue solution was prepared in 0.01 N HCl containing 0.2% NaCl. Standard heparin solution was prepared by 20 mg heparin, diluted to 100 ml with SBF solution. The standard dilutions were between 5 and 40 µg of heparin in the sample. One and one quarter (1.25) ml of toluidine blue solution (0.005 %), and 1.25 ml of in SBF solution were pipetted into test tubes. All the tubes were mixed vigorously by Vortex for 30 s. Next, 2.5 ml of hexane was added to the tubes and they were shaken for another 30 s to separate the heparin-dye complex formed. The aqueous layers of the tubes were sampled and if necessary diluted with SBF. The absorbance at 631 nm was measured within 30 min with Shimadzu UV-Vis-1601 Spectrophotometer.

Silica determination

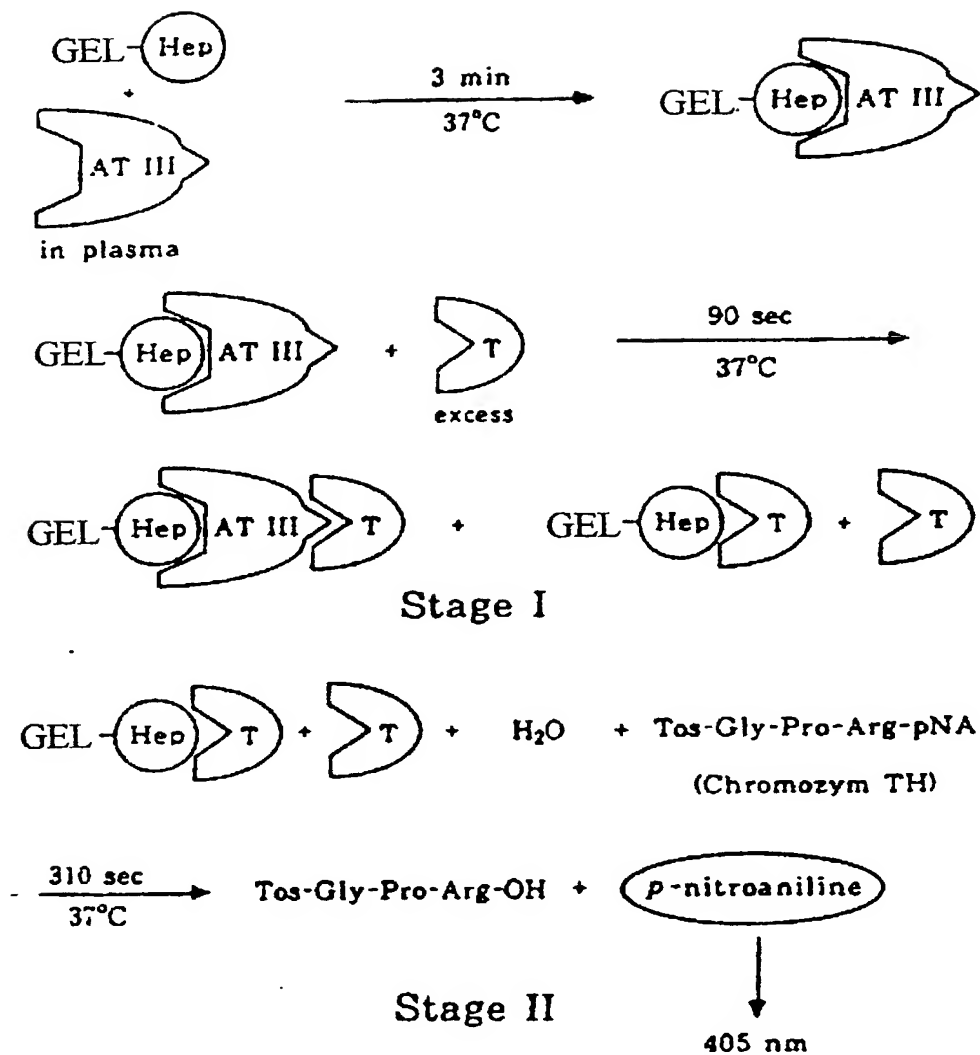
Degradation of the silica xerogel matrix was determined by measuring dissolved Si(OH)_4 as a molybdenum blue complex by UV-spectrophotometer at 820 nm (Koch and Koch-Dedic, 1974).

Thrombin assay

The biological activity of heparin against thrombin formation was evaluated by the chromogenic method (Hall et al., 1984, Han et al., 1989). Heparin forms heparin-antithrombin III (ATIII)-thrombin (T) complex with ATIII in plasma. As illustrated in Scheme 1, the biological activity of heparin can be directly measured if excess amount of thrombin is used to make heparin-ATIII complexes and the amount of used thrombin is determined with Chromozym TH. Thrombin acts as a catalyst in the splitting of paranitroaniline (pNA) from Chromozym TH. The pNA release rate

Platelet poor plasma (57 μ l) was diluted with Tris buffer solution (245 μ l, pH 8.3) and 150 μ l sample solution in a 5 ml test tube. The test tubes were stirred and incubated at 37°C for 3 min. 150 μ l of thrombin solution (8 IU/ml, Sigma T-7009, St. Louis, MO, USA) was added, mixed and incubated for additional 60 s at 37°C. Then 150 μ l Ghromozym TH solution (1.13 mM, previously heated to 37°C, Tos-Gly-Pro-Arg-pNA, Boehringer Mannheim, Mannheim, Germany) was added, mixed and incubated for 310 s at 37°C. The reaction was stopped by adding 450 μ l of 50 % acetic acid. The samples were analyzed spectrophotometrically at 405 nm using a Shimadzu UV-1601 spectrophotometer. Heparin standards between 0.2 and 1.0 IU/ml were done as samples. The relative biological activity was calculated by comparing the thrombin neutralization of immobilized heparin with that of free heparin. The rate of increase in absorbance at 405 nm due to the appearance of the chromophore, p-nitroaniline, is linearly and inversely related to the effective activity by means of standard curve.

Scheme 1



Results

The test results of certain formulations prepared are shown in Table 3.

5

Heparin release from the different formulations examined occurred during the dissolution of the matrix. At the end of dissolution period (96 h), 10 % of the matrix in the tested formulations was dissolved, measured by silica content, and the same amount of heparin was released, suggesting that the heparin release is controlled by matrix erosion. Heparin release from a formulation containing 1 wt-% of heparin in the sol was identical to the rate of the matrix dissolution. Heparin release from the matrix was measured with toluidine blue method and according to silica xerogel matrix erosion studies. This implies that drug release may be described as a process that is controlled mainly by erosion of the matrix. In addition, the porosity of the matrix have an noticeable effect on the dissolution process. Especially in the case of small molecules, drug release is combined process of diffusion and matrix erosion.

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Effect of catalyst

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A model formulation containing 1 wt % heparin in the sol in order to investigate the influence of the used catalyst in the hydrolysis process on the dissolution rate of heparin. Silica xerogel monoliths were prepared using either acetic acid or nitric acid catalyst. At the pH 2.5 the hydrolysis step was faster while nitric acid was used, 45 - 60 min, than the one carried out by using acetic acid, 5 hours. According to the literature (Brinker & Scherer), the rate and extent of the hydrolysis reaction is most influenced by the strength and concentration of the acid catalyst. All strong acid behaved similarly, whereas weaker acid required longer reaction times to achieve the same extent of the reaction. The reaction rate with weaker acid can be accelerated by increasing the used reaction temperature.

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In the present case, the pH of the sol was raised to 4.5-4.8 with NH_4OH after hydrolysis step to avoid the precipitation of heparin. The rate of gel formation, *ie.* the rate of the condensation reactions, is influenced by pH and has a considerable influence on the three dimensional structure of silica network (Brinker & Scherer).

- 5 The gel time is longest near the isoelectric point (IEP) of silica, pH 2, and decreases with increasing pH of the sol (Iler, 1979). Near the IEP there is no electrostatic particle repulsion. Slower kinetics produce linear silica aggregates and more condensed structure but when the pH is raised, gel formation rate increases resulting in more porous structure.

10

Figure 1 shows the cumulative release of heparin versus time for formulations containing 1 weight-% of heparin, calculated on the sol, for xerogels made using nitric acid or acetic acid catalyst. When fitted to zero order model the heparin release was linear with both catalysts. The dissolution rate was 60 % slower from
15 acetic acid catalyzed gels compared to gels catalyzed with nitric acid. This may be due to the higher density of the xerogel prepared by acetic acid catalyst (formulation No. 1, Table 3) compared to that prepared by nitric acid catalyst (formulation No. 6, Table 3).

20 Effect of heparin concentration

- The effect of heparin concentration was studied by using both nitric and acetic acids as the catalyst. The release of heparin from silica xerogel matrix prepared at pH 4.8 (acetic acid catalysed) with the different loads of heparin sodium salt in the silica
25 sol (1, 1.5 and 2 wt %, calculated on the sol) was linear according to zero order kinetics (Figure 2). The release rates of these different matrixes were found to be directly proportional to the drug load of the matrix. Similar releasing profiles, zero order, were observed while nitric acid was used. Correlation between the release rate and the drug load can be used to predict the release rate of heparin from the
30 silica xerogel matrix with the same surface area. The matrix erosion was also linear

and the heparin concentration did not have an influence on the degradation rate of the matrix. These findings are in accordance with our previous paper (Ahola et al., 1999).

5 Effect of organomodified alkoxysilanes

The release rate of biologically active molecules can be influenced by chemical modification of the silica xerogel matrix (Böttcher et al., 1998). Incorporation of organomodified alkoxysilanes into hydrolysis step with TEOS results increasing hydrophobicity of the matrix and changes in porosity. In this study, modification of nitric acid catalyzed sol with co-hydrolysis of TEOS with METES, ETES or DMDES, was carried out. Partial substitution of TEOS with 10 or 25 mol-% of organomodified alkoxysilanes were used. This partial substitution results in more brittle materials. All monoliths were broken during dissolution period which of course have an effect on the release rate. The addition of 10 mol-% organomodified alkoxysilane into the sol did not have any significant effect on the release rate of heparin. The release of heparin was linear according to zero order kinetics from all formulations containing 10 mol-% of organomodified alkoxysilane. When the amount was increased to 25 mol-%, the release behaviour of heparin was better fitted to first order kinetics indicating diffusion controlled process. The release rate of the drug was increased 20 to 40 % when 25 mol-% ETES and DMDES were used. Another reason for the faster releasing rate, besides the brittle structure, can be decreasing possibility to form hydrogen bonds between silica network and heparin.

Table 3

Formulation no.	Dissolution of heparin b=slope (%/h)	Degradation of the matrix	Density (g/cm ³) (SD)
1	r=0.9772 b=0.133	r=0.9964 b=0.101	1.670 (0.012)
2	r=0.9975 b=0.382	r=0.9995 b=0.074	1.675 (0.017)
3	r=0.9980 b=0.512	r=0.9992 b=0.071	1.736 (0.020)
4	r=0.9986 (2-48h) b=1.166	r=0.9971 b=0.089	1.824(0.032)
5	r=0.9466 (2-48h) b=1.853	r=0.9919 b=0.099	1.889(0.007)
6	r=0.9822 b=0.341	r=0.9997 b=0.085	1.635 (0.009)

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It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the specialist in the field that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are

5 illustrative and should not be construed as restrictive.

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1. A composition for controlled release of a biologically active agent from a carrier, wherein the biologically active agent is heparin or a related biologically active acidic polysaccharide and that the carrier is a sol-gel derived silica xerogel, **characterized** in that the xerogel is derived from a tetraalkoxysilane such as tetraethoxysilane (TEOS) and that part of the tetraalkoxysilane is replaced by an organomodified alkoxy silane, preferably an alkylsubstituted alkoxy silane.
2. The composition according to claim 1, **characterized** in that the alkylsubstituted alkoxy silane is methyltriethoxysilane (METES), dimethyldiethoxysilane (DMDES) or ethyltriethoxysilane (ETES).
3. The composition according to claim 1 or 2, **characterized** in that the biologically active agent is heparin in an amount of 5 to 30 weight-% calculated on the air dried xerogel.
4. A method for the preparation of a composition according to any of the claims 1 to 3, **characterized** by the steps of
- a) hydrolysing an alkoxy silane and an organomodified alkoxy silane in the presence of a catalyst,
- b) optionally adjusting the pH to a value suitable for the biologically active agent,
- c) adding the biologically active agent,
- d) allowing the hydroxysilane to polymerize, and optionally
- e) removing water and alcohol formed in the hydrolyzation from the mixture.
5. The method according to claim 4, **characterized** in that the alkoxy silane is a tetraalkoxysilane such as tetraethoxysilane (TEOS).

6. The method according to claim 5, **characterized** in that the organomodified alkoxysilane is an alkylsubstituted alkoxysilane such as methyltriethoxysilane (METES), dimethyldiethoxysilane (DMDES) or ethyltriethoxysilane (ETES).

- 5 7. The method according to claim 4, 5 or 6, **characterized** in that nitric acid or acetic acid is used as catalyst.

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IIFor two-letter codes and other abbreviations, refer to the "Guid-
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ning of each regular issue of the PCT Gazette.(54) Title: NOVEL COMPOSITIONS FOR CONTROLLED RELEASE OF A BIOLOGICALLY ACTIVE AGENT, AND THE
PREPARATION THEREOF(57) Abstract: This invention relates to a composition for controlled release of a biologically active agent from a carrier. The biolog-
ically active agent is heparin or a related biologically active acidic polysaccharide and the carrier is a sol-gel derived silica xerogel.
The xerogel is derived from a tetraalkoxysilane such as tetrethoxysilane (TEOS) and part of the tetraalkoxysilane is preplaced by an
organomodified alkoxysilane, preferably an alkylsubstituted alkoxysilane. The invention also concerns a method for the preparation
of said composition.

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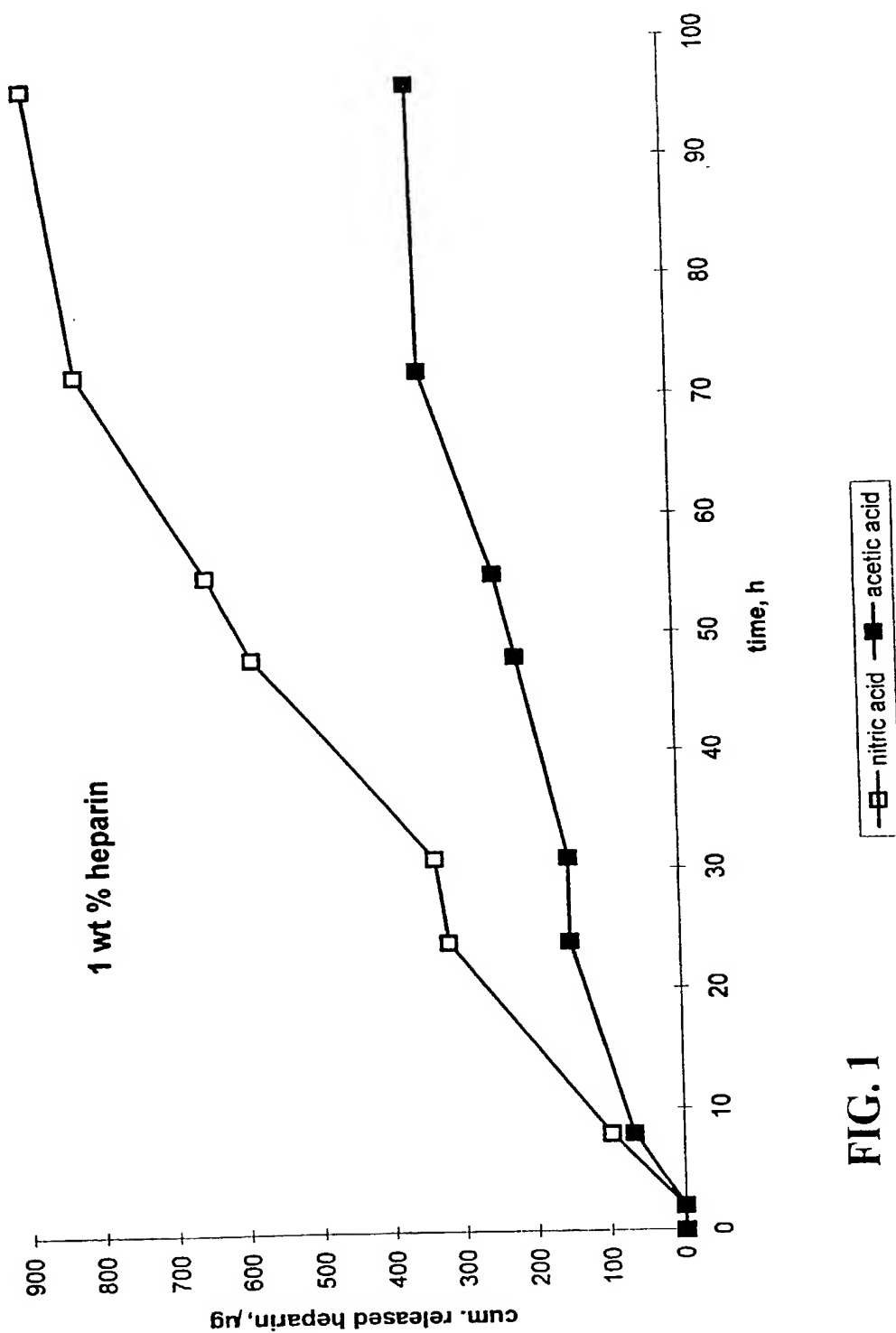


FIG. 1

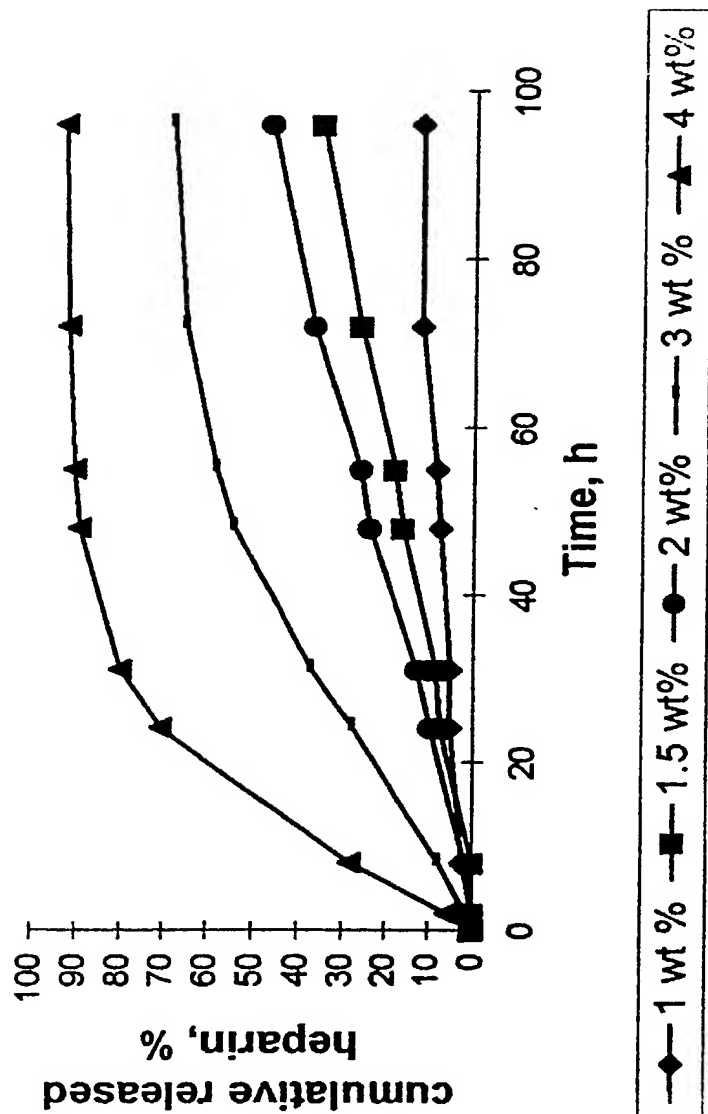


FIG. 2

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Docket No. TUR-125**Declaration For U.S. Patent Application**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
 (INSERT TITLE) NOVEL COMPOSITIONS FOR CONTROLLED RELEASE OF A BIOLOGICALLY ACTIVE AGENT
AND THE PREPARATION THEREOF

the specification of which

(Check one of
1, 2, or 3.)

1. ☐ is attached hereto.
2. ☒ was filed on August 22, 2000 as
International PCT Application Serial No. PCT/FI00/00710
and was amended on _____ (if applicable)
3. ☐ was filed on _____ as
U.S. Application Serial No. _____
and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application for which priority is claimed:

(List prior
foreign
applications.)

19991806
(Number)

Finland
(Country)

25/08/1999
(Day/Month/Year Filed)

Priority Claimed
☒ Yes ☐ No
☐ Yes ☐ No

☐ See attached list for additional prior foreign applications

I hereby claim the benefit under Title 35, United States Code, §120, of any United States application listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56, which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)

(Filing Date)

(Status)

(Application Serial No.)

(Filing Date)

(Status)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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